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Glucose-induced cAMP signaling in *Saccharomyces cerevisiae* is mediated by the CDC25 protein

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Functional mapping of the cell cycle START gene *CDC25* has revealed two domains which are dispensable for viability (germination and growth in glucose media), but are essential for sporulation and differentially involved in glucose-induced cAMP signaling. The transient rise of cAMP is completely prevented by various deletions within the amino-terminal half (α domain) of the CDC25 gene product. In contrast, the deletion of the carboxy-terminal 38 residues (β2 domain) results in a rapid, but persisting, rise of cAMP. Our data suggest that the α domain of the CDC25 protein is involved in glucose signal transduction, whereas the β2 domain is required for downregulating the cAMP control chain.

CDC25 protein; cyclic AMP; Glucose sensing; Signal transduction; Sporulation; (*Saccharomyces cerevisiae*)

1. INTRODUCTION

Glucose has a hormone-like effect on derepressed yeast cells: it induces a rapid, transient increase of the intracellular cAMP level [1–4]. The cAMP signal requires the activity of at least one of the three hexose-phosphorylating enzymes hexokinase 1, hexokinase 2 or glucokinase [5] and the presence of wild type ras proteins [6], and it triggers a phosphorylation cascade leading to the mobilization of storage carbohydrate, the activation of glycolytic enzymes and the inhibition of gluconeogenesis [7]. The most upstream function of the cAMP control pathway has been shown to be coded by the cell division cycle gene *CDC25* [8–11]. The CDC25 protein is required to activate ras proteins, probably by catalyzing the GDP-GTP exchange reaction [9–12], and in addition is involved in the decision between mitosis and meiosis [13,14]. We have recently identified three functionally different domains of the CDC25 protein by gene disruption and deletion experiments [14]. The amino-terminal half (α domain) and the carboxy-terminal 38 residues (β2 domain) are required for sporulation, but not for growth in glucose media, whereas most of the carboxy-terminal half (β1 domain) is essential for viability in the presence of a wild type cAMP control chain. In addition, the α domain is required for storage carbohydrate mobilization and growth on non-fermentable carbon sources.

In this study we demonstrate that the α domain of the CDC25 protein is also required for glucose-induced cAMP signaling, whereas the β2 domain appears to be involved in downregulating the cAMP control chain.

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2. MATERIALS AND METHODS

2.1. *Construction of mutant strains*

The construction of strains HK3, HK4, KL1, KM4, KM10 and KM18 (see table 1) has been described [14,15]. The mutant allele *cdc25-m4* was introduced by deleting a 705 bp *Hpa*I fragment from plasmid p*cdc25-m1* [14]. The deletion m5 was performed by *Hpa*I/*Bgl*II digestion of p*cdc25-m1*, repair of the *Bgl*II single-stranded end and religation. The resulting plasmids p*cdc25-m4* and p*cdc25-m5* were then cleaved with *Sal*I and *Pvu*II, and the *cdc25*-containing fragments were introduced into HK3 by single step replacement. The chromosomal maps of the *cdc25-m4* and *cdc25-m5* alleles were confirmed by genomic

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Table 1

Strains used in this study

HK3	MATa/MATα	*ura3/ura3 his3/+ leu2/+*
KL1b	MATa	*cdc25-1 ura3*
KM4	MATa/MATα	*cdc25-d1::URA3/cdc25-d1::URA3 ura3/ura3 his3/+ leu2/+*
KM10	MATa/MATα	*cdc25-d3::HIS3/cdc25-d3::HIS3 his3/his3 ura3/+ leu2/+*
KM18	MATa/MATα	*cdc25-m2::URA3/cdc25-m2::URA3 ura3/ura3 his3/+ leu2/+*
KM20	MATa/MATα	*cdc25-m4::URA3/+ ura3/ura3 his3/+ leu2/+*
KM21a(b)	MATa(α)	*cdc25-m4::URA3 ura3 his3*
KM22a(b)	MATa(α)	*cdc25-m4::URA3 ura3 leu2*
KM23	MATa/MATα	*cdc25-m4::URA3/cdc25-m4::URA3 ura3/ura3 his3/+ leu2/+*
KM24	MATa/MATα	*cdc25-m5::URA3/+ ura3/ura3 his3/+ leu2/+*
KM25a(b)	MATa(α)	*cdc25-m5::URA3 ura3 his3*
KM26a(b)	MATa(α)	*cdc25-m5::URA3 ura3 leu2*
KM27	MATa/MATα	*cdc25-m5::URA3/cdc25-m5::URA3 ura3/ura3 his3/+ leu2/+*

blotting as described [14]. Haploid *cdc25-m4* and *cdc25-m5* mutants were obtained after sporulation of the heterozygous diploid transformants KM20 and KM24, respectively, and homozygous mutant diploids (KM23 and KM26) were obtained by crossing haploid mutants with opposite mating types and auxotrophic markers. Sporulation efficiency, growth parameters and storage carbohydrate content were determined as described [14].

2.2. *Determination of intracellular cAMP*

Growth and sampling of yeast cells were performed as described by Nakafuku et al. [16]. Cells were grown overnight at 30°C to the stationary phase and were collected by centrifugation. After washing twice with water cells were resuspended in a buffer containing 10 mM Mes (pH 6.0) and 0.1 mM EDTA and were further incubated for 2 h at 30°C under shaking. Glucose was added to a final concentration of 25 mM. After incubation for various periods of time 0.5 ml aliquots were transferred from the culture to a test tube containing 0.5 ml of 10% (w/v) ice-cold trichloroacetic acid and a small amount of glass beads (0.5 mm ∅), and were subsequently frozen in liquid N_2. The samples were thawed on ice, and cell breakage was achieved by vortexing the tubes for 4 min in 30 s intervals at 4°C. To eliminate trichloroacetic acid from the extracts the procedure of Eraso and Gancedo [17] was used. HCl was added to a final concentration of 10 mM, followed by 4 extractions with diethyl ether. The residual ether was removed by N_2 aspiration, the samples were neutralized by addition of 1 M imidazole, and the cAMP content was determined using the Amersham [^{3}H]cAMP radioassay kit following the recommended procedure.

Protein concentration was determined by the method of Bradford [18] using bovine serum albumin as standard.

3. RESULTS

Fig.1A shows the chromosomal map of the *CDC25* wild type allele, as determined by genomic blotting (data not shown). In a previous study [14] we have described the construction and restriction maps of two groups of mutants: in a first group the selectable marker genes *URA3* or *HIS3* were inserted at positions d1 or d2 (*URA3*) or d3 (*HIS3*), resulting in the deletion of 38 (*cdc25-d1*) or 88 (*cdc25-d2*) carboxy-terminal amino acids. The disruption at the central d3 position dissects the *CDC25* gene into two sub-genes, expressing the non-essential amino-terminal half (α domain, see protein map of fig.1C) under the control of *CDC25* promotor, and separately expressing the essential carboxy-terminal half (β domain) under the control of a fortuitous plasmid-encoded element [14].

In a second group of mutants the selectable marker *URA3* was placed at a functionally neutral position downstream of the *CDC25* gene (fig.1B), followed by a tandem repeat of the downstream region required for single-step gene replacement. Various inframe deletions (m2 – m5) were then introduced within the α domain of the coding region, removing amino acid residues 114–348 (m4), 354–876 (m2), 114–876 (m5) or 354–1255 (m3) [19].

All manipulations affecting the β1 domain (including the disruption at d2 and the deletion m3 shown in fig.1) turned out to be lethal, whereas the deletion of β2, the central disruption (d3) and the

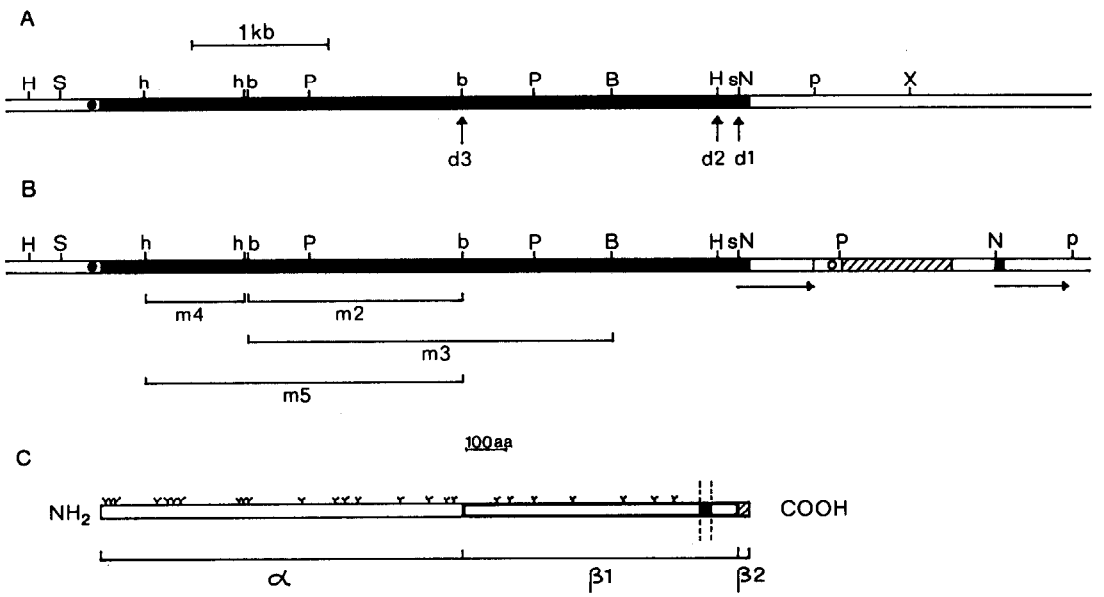

Fig.1. (A) Chromosomal restriction map of the wild type *CDC25* allele. d1 and d2, disruption of the coding region by *URA3*; d3, disruption by *HIS3*. (B) Map of mutant alleles m2–m5 with deletions indicated. Black bar, coding region; shaded bar, *URA3*. Restriction sites: H, *Hin*dIII; S, *Sal*I; h, *Hpa*I; b, *Bgl*II; P, *Pst*I; B, *Bam*HI; s, *Sph*I; N, *Nhe*I; p, *Pvu*II; X, *Xba*I. (C) CDC25 protein map deduced from DNA sequence data [9,19]. Y, potential *N*-glycosylation sites (Asn-X-Ser/Thr); black box, potential membrane-spanning site.

deletions within the α domain did not significantly impair germination and growth in glucose media [14].

The data of fig.2 demonstrate that all non-lethal deletions and disruptions of the *CDC25* gene have a rather striking effect on glucose-induced cAMP signaling. The *CDC25* wild type diploid strain HK3 exhibits a cAMP signal (fig.2A) observed also in other *S. cerevisiae* strains. Typically the starting cAMP level of 0.2–0.3 nmol/mg protein rises to a maximum of 1.5–2 nmol within the first 45 s after glucose addition, and then drops to a final level of about 0.5 nmol/mg protein. All mutant strains having deletions within the α domain (m2, m4, m5) fail to produce a cAMP signal upon addition of glucose, and they maintain a low cAMP level at or below 0.2 nmol/mg protein.

A completely different effect is observed if the carboxy-terminal β2 domain is deleted by inserting the *URA3* gene at position d1, as shown in fig.2B: glucose induces an immediate increase of cAMP comparable to that of the parental *CDC25* wild type strain HK3, but in contrast to the wild type cAMP signal the maximum level is maintained or only slightly reduced within 6 min after induction.

The temperature-sensitive mutant *cdc25-1* produces only very weak signals both at permissive (25°C) and non-permissive temperature (37°C), as shown in fig.2B. The final cAMP level (around

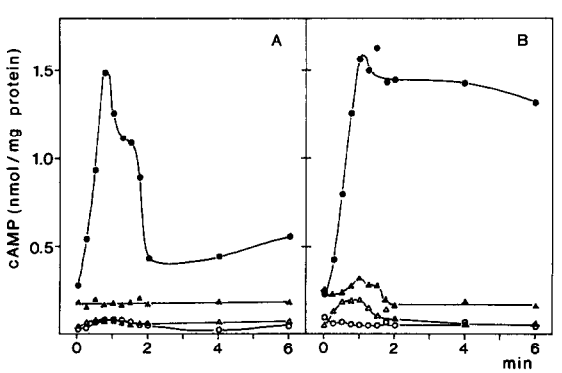

Fig.2. Glucose-induced cAMP signaling in *CDC25* wild type and mutant strains. Glucose was added at zero time to starved cells. (A) Strain HK3 (*CDC25*, ●—●), KM18 (*cdc25-m2*, ○—○), KM21b (*cdc25-m4*, ▲—▲), KM25b (*cdc25-m5*, △—△). (B) KM4 (*cdc25-d1*, ●—●), KM10 (*cdc25-d3*, ○—○), KL1b (*cdc25-1*, ▲—▲) grown at 25°C, or shifted to 37°C for 5 h (△—△).

0.2 nmol/mg protein in cells grown at 25°C) is already much lower than the wild type level, and is further reduced after arrest at 37°C.

4. DISCUSSION

Our data clearly demonstrate that the *CDC25* gene product is involved in glucose-induced cAMP signaling, and that manipulations at three operationally defined domains of the CDC25 protein have quite different effects on glucose signal transduction. A previous study has shown that these domains differentially control also other phenotypical parameters, such as growth on fermentable and non-fermentable carbon sources, mobilization of storage carbohydrates and sporulation [14]. Deletions within the amino-terminal half (α domain) do not affect viability and growth in glucose media, but completely abolish the glucose-induced transient rise of cAMP, indicating that a functional glucose signal transduction chain is not a prerequisite for mitotic growth in glucose media. The same group of mutants (*cdc25* alleles m2, m4 and m5, see protein map of fig.1C) fails to grow on non-fermentable carbon sources, to sporulate (if in a diploid state) and to mobilize the storage carbohydrates trehalose and glycogen. Therefore, the α domain appears to be required not only for glucose signal transduction, but also for starvation response and initiation of meiosis, probably by sensing the nutritional environment.

The main part of the carboxy-terminal half (β1 domain) of the CDC25 protein is essential for viability (germination and growth in glucose media), because deletions and disruptions affecting the β1 domain (such as m3 and d2, see fig.1) are lethal in strains having a wild type cAMP control chain [14]. Furthermore, the conditional-lethal *cdc25-1* mutation [8] is located within this domain, because multicopy plasmids containing the β1 fragment complement the temperature-sensitive lesion [9,10,15,19,20]. Our data (fig.2B) demonstrate that the β1 domain is also involved in glucose signal transduction, since the *cdc25-1* mutation strongly reduces the cAMP signal, even at permissive temperature.

The β1 domain is required for the activation of adenylate cyclase via activation of ras proteins, probably by catalyzing the GDP-GTP exchange on *ras1* or *ras2* [9,12], and at least one functional *ras* gene is required for glucose-induced cAMP signaling [6]. The β2 domain is not required for growth on fermentable and non-fermentable carbon sources and for storage carbohydrate mobilization, but for sporulation [14]. The data of fig.2B demonstrate that the β2 domain is dispensable for the glucose-induced hyperactivation of *ras*-adenylate cyclase, but is required for down-regulating adenylate cyclase. This finding immediately supports the recently proposed feedback control loop of the cAMP signaling system in yeast [11]. One of the components of the cAMP control chain, the ras2 protein, is downregulated by phosphorylation through the catalytic subunit of cAMP-dependent protein kinase [21], and a similar feedback loop might operate to downregulate the CDC25 protein, in analogy to the homologous desensitization of hormone receptors [22,23]. The most straightforward idea would be a downregulation by cAMP-dependent phosphorylation of a β2 threonine or serine residue, although a more indirect role of β2 is also possible. A special comment is deserved to explain the phenotype of the *cdc25-d3* mutant, where the two halves of the gene are separately expressed as α and β subunits. Obviously this mutant shares all properties with mutants having deletions in the α domain (m2, m4, m5), including the loss of glucose-induced cAMP signaling and starvation response, although the α domain is expressed under the control of the *CDC25* promotor [14]. Recent complementation experiments with heterozygous *cdc25* mutant diploids favour the idea that the α domain has to be attached to a β1 region including a potential membrane-spanning domain, in order to be carried to its functional site. Since the *CDC25* gene product is the most upstream function known to be involved in the activation of adenylate cyclase [9,10] and in the decision between mitosis and meiosis [13,14], it is tempting to consider the CDC25 protein as a receptor-like transmembrane protein having an extracellular nutrient-sensing domain or subunit.

On the other hand, our data do not rule out an intracellular glucose-sensing function of the CDC25 protein. The cAMP signal would then be triggered by other glucose-sensing surface proteins, such as hexose transporters [5,24]. However, cAMP signaling appears to be independent of the

SNF3 gene function [6] encoding a hexokinase-dependent high affinity glucose transporter [25,26], and is not affected by disrupting a recently cloned gene controlling low affinity glucose uptake (our unpublished data). Therefore, the relation between glucose transport and glucose sensing remains to be established.

REFERENCES

[1] Purwin, C., Leidig, F. and Holzer, H. (1982) Biochem. Biophys. Res. Commun. 107, 1482–1489.
[2] Mazon, M.J., Gancedo, J.M. and Gancedo, C. (1982) Eur. J. Biochem. 127, 605–608.
[3] Thevelein, J.M. (1984) Arch. Microbiol. 138, 64–67.
[4] Thevelein, J.M. and Beullens, M. (1985) J. Gen. Microbiol. 131, 3199–3209.
[5] Beullens, M., Mbonyi, K., Geerts, L., Gladines, D., Detremerie, K., Jans, A.W.H. and Thevelein, J.M. (1988) Eur. J. Biochem. 172, 227–231.
[6] Mbonyi, K., Beullens, M., Detremerie, K., Geerts, L. and Thevelein, J.M. (1988) Mol. Cell. Biol. 8, 3051–3057.
[7] Holzer, H. (1984) in: Molecular Aspects of Cellular Regulation, vol.3 (Cohen, P. ed.) pp.143–154, Elsevier, Amsterdam.
[8] Hartwell, L.H. (1974) Bacteriol. Rev. 38, 164–198.
[9] Broek, D., Toda, T., Michaeli, T., Levi, L., Birchmeier, C., Zoller, M., Powers, S. and Wigler, M. (1987) Cell 48, 789–799.
[10] Robinson, L.C., Gibbs, J.B., Marshall, M.S., Sigal, I.S. and Tatchell, K. (1987) Science 235, 1218–1221.
[11] Nikawa, J., Cameron, S., Toda, T., Ferguson, K.M. and Wigler, M. (1987) Genes Dev. 1, 931–937.
[12] Daniel, J., Becker, J.M., Enari, E. and Levitzki, A. (1987) Mol. Cell. Biol. 7, 3857–3861.
[13] Shilo, V., Simchen, G. and Shilo, B. (1987) Exp. Cell. Res. 112, 241–248.
[14] Munder, T., Mink, M. and Küntzel, H. (1988) Mol. Gen. Genet. 214, 271–277.
[15] Lisziewiecz, J., Godany, A., Förster, H.H. and Küntzel, H. (1987) J. Biol. Chem. 262, 2549–2553.
[16] Nakafuku, M., Obara, T., Kaibuchi, K., Miyajima, I., Miyajima, A., Itoh, H., Nakamura, S., Arai, K.I., Matsumoto, K. and Kaziro, Y. (1988) Proc. Natl. Acad. Sci. USA 85, 1374–1378.
[17] Eraso, P. and Gancedo, J.M. (1984) Eur. J. Biochem. 141, 195–198.
[18] Bradford, M.M. (1976) Anal. Biochem. 72, 248–254.
[19] Camonis, J.H., Kalékine, M., Gondré, B., Garreau, H., Boy-Marcotte, E. and Jacquet, M. (1986) EMBO J. 5, 375–380.
[20] Martegani, E., Baroni, M.D., Frascotti, G. and Alberghina, L. (1986) EMBO J. 5, 2363–2369.
[21] Resnick, R.J. and Racker, E. (1988) Proc. Natl. Acad. Sci. USA 85, 2474–2478.
[22] Sibley, D.R., Benovic, J.L., Caron, M.G. and Lefkowitz, R.J. (1987) Cell 48, 913–922.
[23] Levitzki, A. (1988) Trends Biochem. Sci. 13, 298–301.
[24] Eraso, P. and Gancedo, J.M. (1985) FEBS Lett. 191, 51–54.
[25] Bisson, L.A., Neigeborn, L., Carlson, M. and Fraenkel, D.G. (1987) J. Bacteriol. 169, 1656–1662.
[26] Celenza, J.L., Marshall-Carlson, L. and Carlson, M. (1988) Proc. Natl. Acad. Sci. USA 85, 2130–2134.